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Citation

Singer, Hannah M., Bobby Y. Reddy, and Marc E. Grossman. 2017. "Touch preparation for the rapid diagnosis of disseminated aspergillosis." *JAAD Case Reports* 3 (3): 202-204. doi:10.1016/j.jdc.2017.02.006. <http://dx.doi.org/10.1016/j.jdc.2017.02.006>.

Published Version

doi:10.1016/j.jdc.2017.02.006

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Touch preparation for the rapid diagnosis of disseminated aspergillosis



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Key words: aspergillosis; diagnostic technique; fungal stain; medical dermatology; touch preparation.

INTRODUCTION

Aspergillus sp, *Candida* sp, and zygomycoses can disseminate rapidly in an immunocompromised host, causing significant morbidity and mortality.¹ Serum testing, tissue biopsy, and culture are used to confirm a suspected diagnosis, but waiting for results may delay initiation of life-saving treatment. We report a case of disseminated aspergillosis presenting with a cutaneous nodule. A rapid diagnosis was made on direct visualization of the fungal organisms on a touch preparation from a skin biopsy, and treatment was immediately initiated.

CASE

A 67-year-old man with advanced B-cell non-Hodgkin lymphoma on high-dose cytosine arabinoside chemotherapy was admitted for worsening multifocal pneumonia. During a previous admission, he completed a 10-day course of intravenous antibiotics for pneumonia. Six days after discharge, he was readmitted for dyspnea. The dermatology department was consulted for asteatotic dermatitis.

On examination, a single 1.5-cm violaceous, nontender nodule with surrounding erythema overlying the right knee (Fig 1) was described by the patient as a "bruise" from bumping his leg several days prior. Given the patient's worsening pneumonia and immune compromised status and the vivid hue of this nodule, clinical suspicion was high for a disseminated fungal infection. At the time of skin biopsy, the specimen was noted to be unusually friable. A touch preparation was performed by gently smearing the specimen across a clean glass slide. The

Abbreviation used:

KOH: potassium hydroxide

smear specimen was air dried on the slide for 5 minutes then stained with chlorazol black E, an agent that targets the polysaccharide chitin found in fungal cell walls. Filamentous septate branching hyphae were visible on direct microscopy 5 minutes after staining (Fig 2). The patient was started on intravenous amphotericin the same evening that dermatology was consulted.

The following day, hematoxylin-eosin staining of the skin biopsy section showed dense collections of branching hyphae. Gomori methenamine silver stain (Fig 3) highlighted acutely branching hyphae and septae, morphologic features consistent with *Aspergillus* spp. Six days later, cultures grew *Aspergillus flavus*.

DISCUSSION

Opportunistic invasive fungal infections are a frequent cause of morbidity and mortality in immunocompromised patients. Prolonged neutropenia or defects in neutrophil function predispose hosts to *Aspergillus*, *Candida*, and zygomycoses. Newer chemotherapy regimens and the increased use of fluconazole prophylaxis are factors contributing to the increase in *Aspergillus* infections relative to *Candida*.¹

Cutaneous aspergillosis can be a primary disease or secondary manifestation of systemic infection. Primary cutaneous aspergillus infections are often

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Funding sources: None.

Conflicts of interest: None declared.

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JAAD Case Reports 2017;3:202-4.

2352-5126

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<http://dx.doi.org/10.1016/j.jidcr.2017.02.006>

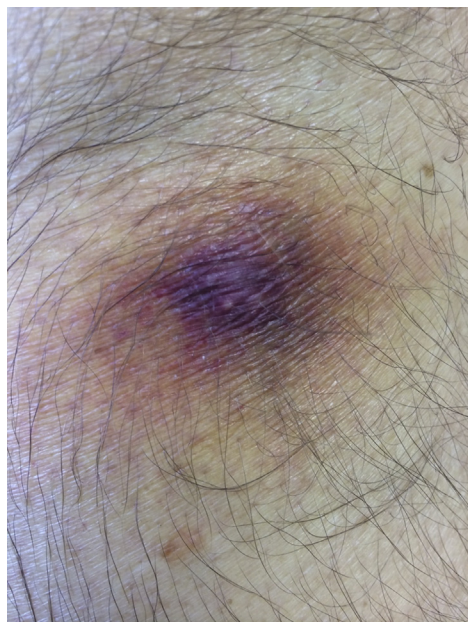


Fig 1. Violaceous nodule noted on patient's right knee.

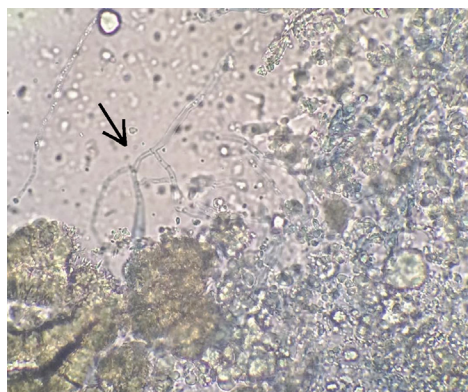


Fig 2. Touch preparation shows filamentous, septate hyphae (arrow) visible on direct microscopy 5 minutes after staining with Chlorazol Black E.

traced to nosocomial sources. The skin findings in primary cutaneous aspergillosis are usually tender, erythematous to purpuric macules or papules that evolve into violaceous plaques. Hemorrhagic bullae may form, and the blister roof can be examined with potassium hydroxide (KOH) for a rapid presumptive diagnosis. *A. flavus* and *Aspergillus niger* are the most common causes of primary cutaneous aspergillosis.²

Secondary cutaneous aspergillosis can arise after hematogenous spread from a primary lung infection, as in this case, or from underlying infected respiratory structures such as the nose, sinus, or orbits. Cutaneous findings are reported in 1% to 5% of patients with systemic aspergillosis,³ but any skin changes in an ill immunocompromised patient on antibiotics should be urgently assessed for an

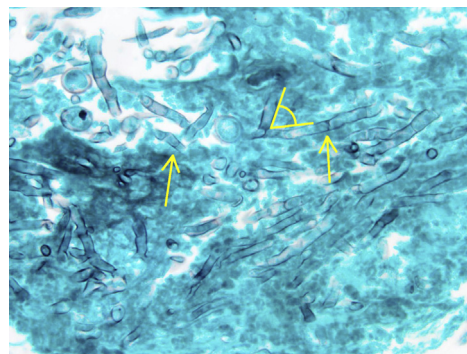


Fig 3. Gomori methenamine silver stain highlighting the septae (arrows) and acute angle, dichotomously branching hyphae.

opportunistic fungal infection. Lesions may initially present as nontender erythematous or purpuric macules, papules, or nodules and subsequently become hemorrhagic, ulcerated, or necrotic or undergo formation of an abscess. Clusters of nodules can arise regionally as erythematous, cellulitic plaques or verrucous, vegetative, necrotic plaques with crust. Skin biopsy findings show angioinvasion, thrombosis, and an inflammatory infiltrate within the deep dermis and subcutaneous fat. Most disseminated *Aspergillus* infections are caused by *Aspergillus fumigatus*.²

In critically ill, immunocompromised patients, in whom aspergillosis is most often suspected, the most accurate tests, such as bronchial alveolar lavage, may not be feasible. Cultures can take several days to grow, and a negative culture does not rule out a fungal etiology. Computed tomography findings for pulmonary aspergillosis are not specific, as other bacterial or fungal infections may look similar. Serum testing with Galactomannan and β -glucan assays can be helpful; however, false-positive results may occur in the presence of certain antibiotics, other fungi, or bacteria.^{4,5} Other noninvasive methods are being actively investigated, including polymerase chain reaction, lateral flow devices, volatile organic compounds, *Aspergillus*-derived siderophores, and mass spectrometry, but these have not yet been validated in large, clinical trials.⁶

The touch preparation for direct microscopy of biopsied skin may show fungal organisms that can expedite the initiation of appropriate treatment. Although few cases are reported in the literature (Table I),^{7,8} a touch preparation slide takes only a few minutes and may be a useful routine practice when taking skin biopsy specimens from immunocompromised patients. When secondary cutaneous invasive aspergillosis is suspected, it is crucial to ensure that tissue for touch preparation is

Table I. Case reports of touch preparations from skin biopsy specimens for diagnosis of fungal infections

Organism	Lesion appearance	Host factors	Stain
<i>Candida tropicalis</i> ⁷	Erythematous papules with central white spots	Acute myelogenous leukemia, neutropenia	KOH and Gram stain
<i>Candida guilliermondii</i> , <i>Candida tropicalis</i> , and <i>Candida albicans</i> ⁷	Erythematous and purpuric papules and nodules with necrotic centers	Acute myelogenous leukemia, neutropenia	KOH
<i>Rhizopus arrhizus</i> ⁸	"Bull's-eye infarct" of coalescing annular, necrotic, plaques	Chronic lymphocytic leukemia, splenectomy	PAS
<i>Cryptococcus</i> ⁹	Erythematous, tender nodules	Liver transplant recipient	Eosin and methylene blue (Quik-Dip)
<i>Rhizopus oryzae</i> ¹⁰	Necrotic ulceration with violaceous borders and surrounding erythema	Liver transplant recipient, <i>Cytomegalovirus</i> viremia, renal failure	KOH and Parker blue ink (Swartz Lamkins fungal stain)
<i>Aspergillus flavus</i> (Current case)	Single violaceous nontender nodule with surrounding erythema	B-cell non-Hodgkin lymphoma	Chlorazol Black E

KOH, Potassium hydroxide; PAS, periodic acid–Schiff.

recovered from the base of the biopsy section. Skin manifestations result from fungal invasion of subcutaneous vessels and fat.

A variety of histologic stains can aid in identifying fungal organisms on direct microscopy of touch preparations. Many stains target chitin in fungal cell walls, although there are differences in the drying time and specificity of components stained. KOH may be used to help clear the specimen of debris, and other stains can be added after application of KOH. Chlorazol Black E, used in this case, can be applied directly to the touch preparation tissue smear with staining sufficient to distinguish fungal elements in as little as 5 minutes. A staining procedure designed for blood smears marketed as Quik-Dip (Mercedes Medical, Sarasota, FL) was used on a touch preparation to diagnose cutaneous *Cryptococcal* infection.⁹ Parker blue ink as a component of Swartz Lamkins fungal stain has been reported in touch preparations for cutaneous zygomycosis.¹⁰ Grocott and Gomori methenamine silver stains, hematoxylin-eosin stain, and periodic acid-Schiff with diastase are all routinely used in processed tissue samples and are necessary for confirmation of any presumptive diagnosis based on touch preparation. A simple technique using a spatula and tape has also been described for quickly mounting intact fungal organisms from a tube culture onto a slide for microscopic examination.¹¹

CONCLUSIONS

Touch preparation and direct microscopy of stained tissue may show the presence of fungal

organisms and allow for rapid presumptive diagnosis and initiation of therapy while confirmatory tests are being processed in the acutely ill immunocompromised patient with unexplained skin lesions.

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